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Xeroderma pigmentosum-Cockayne syndrome complex: a further case

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Abstract

We report on a male patient born to healthy, first cousin, Moroccan parents. During the pregnancy growth retardation was observed. Birth weight, length, and OFC were all well below the 3rd centile. Facial anomalies, microphthalmia, cleft palate, small penis, and flexion contractures of large joints were noted. Cerebral MRI showed dysmyelination. The clinical course was characterised by feeding difficulties, growth failure, lack of development, photosensitivity, and death at 7 months. The main differential diagnoses were COFS syndrome and early onset Cockayne syndrome (CS). UV exposure of cultured fibroblasts showed inhibition of nucleic acids synthesis. Further DNA repair studies showed extreme cellular sensitivity to UV and xeroderma pigmentosum (XP)-like defective nucleotide excision repair (NER), which in combination with the clinical symptoms indicated the very rare XP-CS complex. Complementation analysis showed that the XPG gene is affected in this patient. In cases suspected of having COFS syndrome and early onset CS, extensive DNA repair studies are needed to reach the definitive diagnosis, thereby allowing reliable genetic counselling and prenatal diagnosis.

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Key words: COFS syndrome; Cockayne syndrome; XP-CS complex.

To date seven independent cases of the rare xeroderma pigmentosum (XP)-Cockayne syndrome (CS) complex have been reported. Two belong to the XP complementation group B,¹⁻³ two to D,^{4,5} and three to group G.⁶⁻⁸ We report on a further case and stress the importance of performing extensive DNA repair studies in cases suspected of having COFS syndrome (MIM 214150) and early onset CS (MIM 216400) in order to reach a correct diagnosis, thereby allowing reliable genetic counselling and prenatal diagnosis.

Methods

CASE REPORT

The male proband is the third child of healthy, first cousin, Moroccan parents. The pregnancy was complicated by growth retardation and delivery was induced at 38 weeks' gestation. Birth weight was 1770 g, length 40.5 cm, and OFC 31 cm (all <3rd centile). Apgar scores at one and five minutes were 9 and 10 respectively.

On examination facial anomalies were observed including small palpebral fissures, prominent nasal bridge, small mouth, posteriorly rotated ears, micrognathia, and cleft palate. He had flexion contractures of the large joints and clenched hands with overlapping of the second finger over the third and the fifth finger over the fourth. Micropenis and pigmented spots on the trunk and right leg were noted (fig 1). Feeding difficulties were present from birth, necessitating tube feeding and admission to hospital (except for about two weeks) until he died at the age of 7 months. The clinical course was further characterised by the development of hepatomegaly, growth failure, and an almost complete absence of motor and mental development. Retrospectively, one event of sun exposure with a hypersensitive skin reaction was noted; no other skin changes were observed. He reacted to sound, but not to light stimuli. At the age of 7 months his weight was 4300 g, length 51 cm, and OFC 36 cm (all <3rd centile). Necropsy was not performed. Family history was not contributory.

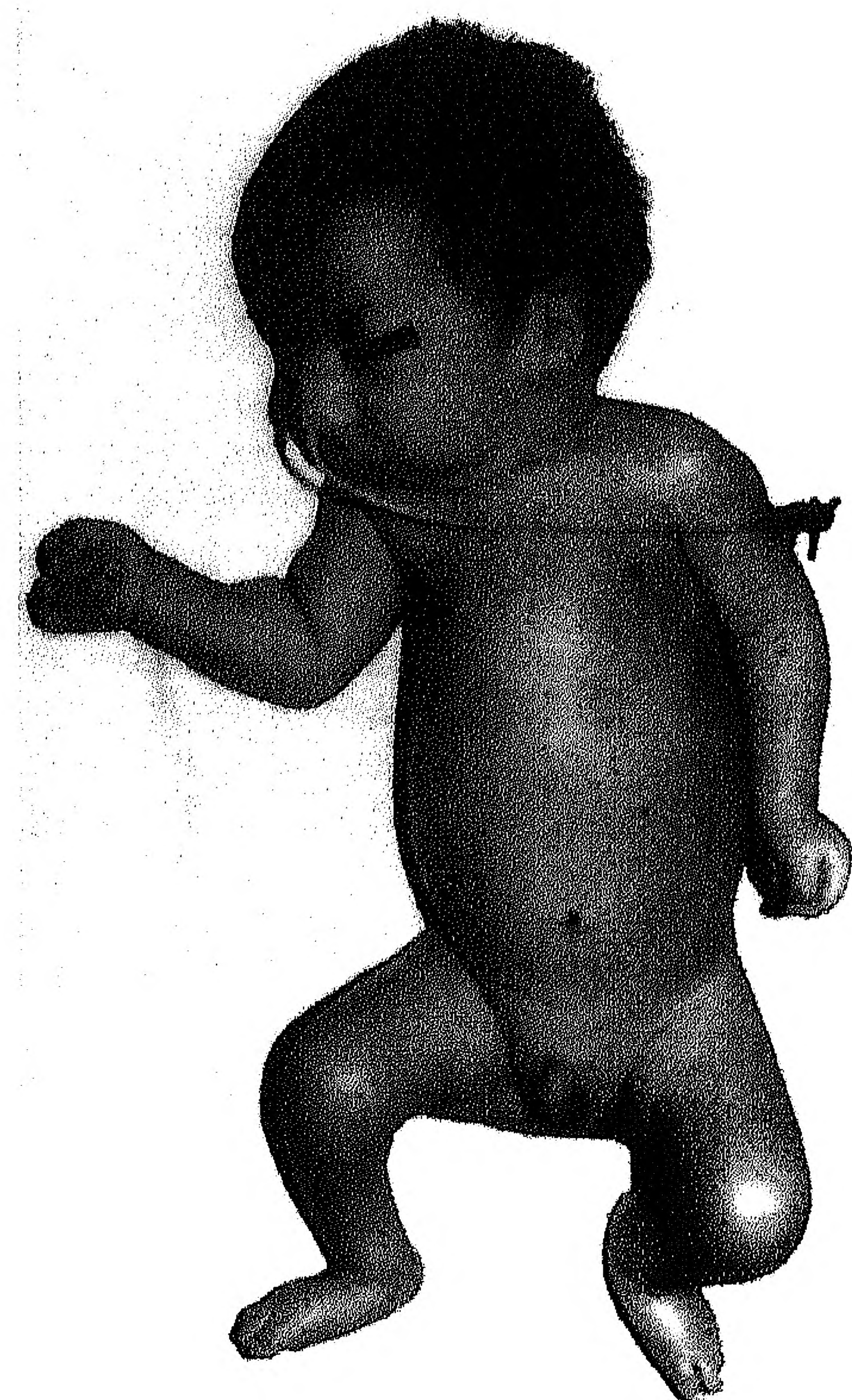


Figure 1 Note the prominent nasal bridge, posteriorly rotated ears, micrognathia, clenched hands with overlapping fingers, micropenis, and pigmented spot on the trunk.

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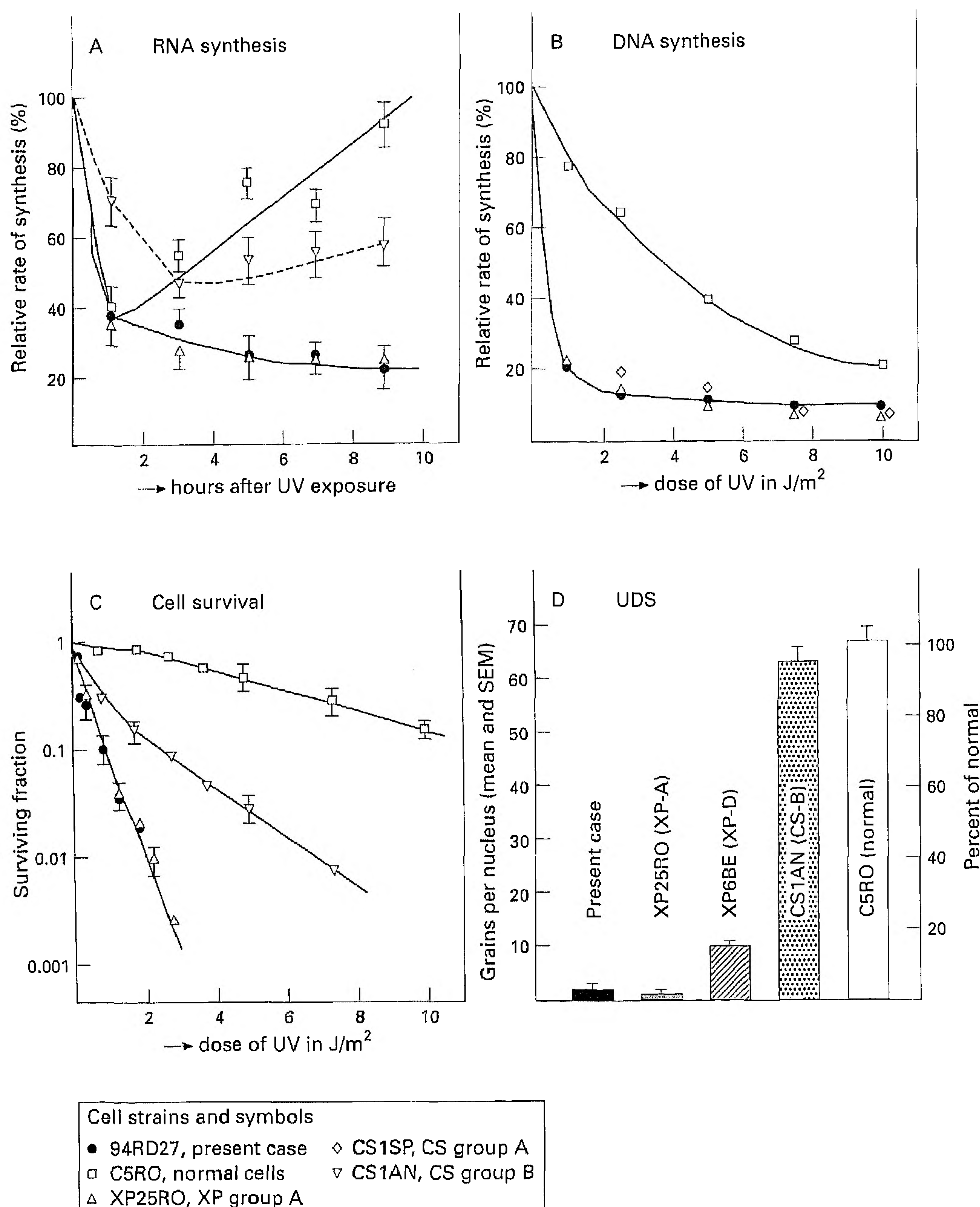


Figure 2 (A) Kinetics of RNA synthesis after 4 J/m² of UV light. (B) UV dose response of DNA synthesis rate at 16 hours after irradiation. (C) Cell survival assay. (D) Unscheduled DNA synthesis (UDS). If not too small for drawing, bars are indicated in the survival curves to represent standard errors from duplicate dishes.

DNA REPAIR ASSAYS

RNA synthesis

Coverslip cultures of fibroblasts were exposed to 4 J/m² of 254 nm UV light and labelled for one hour with 3H-uridine at the indicated times after irradiation. Cultures were fixed and processed for autoradiography. RNA synthesis rates were measured by counting grains over 40 to 50 nuclei and expressed as percentages of unirradiated control cultures.

DNA synthesis

Fibroblast cultures were prelabelled with 14C-thymidine overnight, exposed to various doses of UV, incubated for two hours with 3H-thymidine at 16 hours after irradiation, and harvested. The ratio of 3H to 14C radioactivities, assessed by scintillation counting, was taken as

a measure of the rate of DNA replication and expressed as a percentage of the ratio in unirradiated cells.

Cellular sensitivity

Sparsely seeded petri dish cultures were exposed to UV and allowed to grow for another four to five days. Then the number of proliferating cells in each dish was estimated by scintillation counting of the radioactivity incorporated during a two to three hour pulse labelling with 3H-thymidine. Cell survival was expressed as the ratio of irradiated over unirradiated cells. The results of this simplified UV survival assay fully agree with those obtained in the more standard clonogenic assays (Jaspers and Raams, unpublished data).

Unscheduled DNA synthesis

Coverslip cultures of fibroblasts were exposed to 10 J/m² of 254 nm UV light and subsequently labelled for two hours with 3H-thymidine. After fixation and processing for autoradiography, grains were counted above the nuclei of 50 non-S-phase cells, as described in detail previously.^{4,7}

Complementation analysis

After preloading the cytoplasm with plastic microspheres of different sizes, two cell strains were fused using Sendai virus, seeded on coverslips, and assayed for unscheduled DNA synthesis one day later. Grains were counted above 50 nuclei in homodikaryons and heterodikaryons, discriminated by their content of cytoplasmic beads, as described in detail previously.⁷

Results

G banded chromosomes of peripheral lymphocytes were normal. Cardiac and abdominal ultrasound showed no abnormalities. Radiology of the skeleton was normal. Cerebral CT scan showed enlarged ventricles and cerebral MRI dysmyelination and cerebellar hypoplasia; calcifications were not seen. On repeat ophthalmological examination microphthalmia was seen, but no cataract or retinopathy. Cortical blindness was suspected. Because early onset CS was in the differential diagnosis, DNA repair studies were initiated. Inhibition of nucleic acid synthesis after UV exposure is the usual parameter tested in cases of CS. During the first nine hours after UV irradiation, the patient's cultured fibroblasts completely failed to recover their rate of RNA synthesis, under conditions where normal control cells returned to normal levels (fig 2A). Consistent with this result, DNA replication, measured at 16 hours after exposure to graded UV doses, was abnormally depressed as well (fig 2B). A cell survival assay confirmed UV hypersensitivity, which was more severe than usually observed in CS patients' cells (fig 2C). As the failure to restore RNA and DNA synthesis after UV irradiation, as well as the decreased cell survival, is not only associated with CS but also with XP (and XP-CS complex), DNA repair characteristics were studied further. UV induced unscheduled DNA synthesis (UDS), measured by autoradiography, was less than 4% of that in normal cells (fig 2D), indicating a strongly impaired overall NER, which is characteristic of XP but not of CS. For further genetic characterisation, the patient's cells were fused to representatives from various XP complementation groups. In the heterokaryons, UDS was restored to normal levels after fusion with cells from XP groups B and D (compare to fig 2D), but not with group G cells (table). This result shows the involvement of the XPG (ERCC5) gene, which is believed to specify the 3' strand incision function in the NER process.⁹

Complementation analysis of defective NER

| Fusion with cell strain | Grains per nucleus: mean (SEM) | | |
|----------------------------|--------------------------------|------------|-----------------|
| | Homodikaryons | | Heterodikaryons |
| | Patient | Partner | |
| XPCS1BA (XP-B) | 2.2 (0.2) | 7.0 (0.6) | 58.7 (2.3) |
| XP6BE (XP-D) | 2.5 (0.3) | 13.0 (0.8) | 65.6 (2.3) |
| XP3BR (XP-G) | 2.5 (0.3) | 3.5 (0.4) | 3.6 (0.3) |

Discussion

There is considerable clinical overlap between early onset CS and COFS syndrome and Lowry¹⁰ and Patton *et al*¹¹ have previously pointed to their close similarity. The sibs which Lowry *et al*¹² described as a new syndrome were classified by some^{11,13} as an example of early onset CS and by others^{14,15} as an example of COFS syndrome. Because of the absence of cerebral calcifications, cataract, and retinopathy, our initial diagnosis was COFS syndrome with early onset CS as a differential diagnosis. Detailed analysis of DNA repair showed the final (genetic) diagnosis to be XP-G. This underlines the importance of extensive DNA repair studies in cases suspected of having early onset CS and COFS syndrome in order to reach a correct diagnosis. XP and CS both feature skin hypersensitivity to sunlight and belong to the nucleotide excision repair (NER) syndromes. Characteristically, CS has a defect in transcription coupled NER, whereas most XP groups are affected in both transcription coupled and "global" NER.¹⁶ Our case is the ninth patient with symptoms of CS belonging to an XP complementation group. Robbins¹⁷ proposed that this XP/CS combination was a distinct clinical entity and called it "XP-CS complex". The five patients from the four kindreds within complementation groups B and D have a combination of features of both XP and CS, whereas three others belonging to XP-G have virtually only CS. Our patient did not show overt XP symptoms. However, it is quite possible that his early demise and stay in hospital for almost 7 months may have prevented the development of XP skin manifestations, like atrophic skin with hyper- and hypopigmented spots in sun exposed areas. Our report concerns the eighth patient with NER deficiency belonging to XP group G. In 1993 Vermeulen *et al*¹⁷ reviewed seven cases, including those described earlier as cases with severe early infantile CS.⁶ Clinical variability within XP-G ranges from classical XP to early onset CS. Recently, Nospikel and Clarkson¹⁸ detected point mutations in the XPG (ERCC5) genes of one XP-G patient with a mild XP phenotype. Mutational analysis in the other seven XP-G cases may indicate whether there is a predictable genotype–phenotype correlation. This analysis is currently under way in our patient and other patients with the XP-CS phenotype⁸ (S G Clarkson, personal communication).

Based on the finding that the XPB and XPD gene products are part of the basal transcription factor TFIIH, it has been hypothesised that many of the symptoms of the XP-CS complex are caused by subtle deficiencies in basal transcription.¹⁶ In this hypothesis, the clinical vari-

ability within XP-G suggests that the XPG protein has some relation to basal transcription as well; however, direct enzymological support of this notion could not be obtained so far and has to await the development of more sophisticated in vitro repair/transcription assays. In conclusion, we report on a patient with developmental defects and failure to thrive suggestive of COFS syndrome or early onset CS, where cellular studies indicate a defect in the XP-G gene. We stress the importance of performing extensive DNA repair in cases suspected of having COFS syndrome and early onset CS.

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